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# CHANGES IN THE FRACTIONAL COMPOSITION OF THE SERUM PROTEINS OF MICE AND GUINEA-PIGS ON IMMUNIZATION WITH TYPE A CLOSTRIDIUM BOTULINUM TOXOIDS\*

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DURING the process of immunization of animals there are changes in the composition of the protein fractions of the serum. Thus, it was found that on immunization of mice with *Clostridium botulinum* antigen there was an increase in the  $\gamma$ -globulin fraction and a new, T-globulin component appeared (van der Scheer et al., 1940).

We were interested in the changes occurring in the composition of the serum proteins of laboratory animals—mice and guinea-pigs—on immunization with *Clostridium botulinum* toxoids and also the connexion between these changes and the antigenicity of the toxoids.

To study changes in the serum proteins we used electrophoretic fractionation on filter paper (the fractionation technique was described in our paper published in 1956). It should be emphasized that electrophoretic fractionation was only used when the sera showed no signs of haemolysis, since haemoglobin has an electrophoretic mobility approximating that of certain protein factors, and when the electrophoregram is stained it may adsorb part of the stain, altering the true significance of the quantitative relationships of the serum protein fractions as shown by quantitative analysis.

Immunization was carried out with normal and Cellophane† type A toxoids.

Mice weighing 16–18 g were immunized intraperitoneally with 0·5 ml of Cellophane toxoid adsorbed on aluminium phosphate. In two-dose immunization

TABLE I. CHANGES IN THE FRACTIONAL COMPOSITION OF SERUM PROTEINS OF MICE IMMUNIZED WITH ADSORBED CELLOPHANE TYPE A *Clostridium botulinum* TOXOID (AVERAGE FIGURES)

Examination	Time of immunization	Composition of protein fractions (%)						
		Globulins						Albumin-globulin ratio
		Albumin	$\alpha_1$	$\alpha_2$	$\beta$	$\gamma$		
Single dose	Before immunization	57	6·9	8·4	18	9·7	1·33	0·86
	After immunization	46·3	6·7	6·8	21·5	18·7		
Two doses	Before immunization	56·3	7·5	7·8	18·3	10·1	1·29	0·64
	After immunization	39·1	6·5	7·2	21·7	25·2		

\* Zh. mikrobiol., epidemiol. immunobiol. No. 12, 95–98, 1958.

† The Cellophane toxoids were obtained from the toxins formed when cultured in Cellophane sacs.

a 2nd injection of 0.5 ml of adsorbed toxoid was given 20 days after the first. Most of the immunized mice acquired resistance to 2 MLD of *Clostridium botulinum* toxin. The blood of the mice was examined before immunization and after 1 and 2 doses of toxoid. To take the blood a mouse was decapitated, the amount of serum thus obtained from 1 mouse being quite sufficient for examination.

Analysis of the electrophoregrams showed that there were no qualitative differences in the serum protein fractions before and after immunization: in both cases albumin and  $\alpha_1$ -,  $\alpha_2$ -,  $\beta$ - and  $\gamma$ -globulins could be differentiated. There were, however, considerable quantitative differences in the composition of the serum protein fractions before and after immunization.

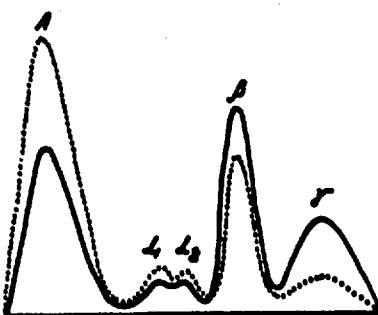


FIG. 1. Fractional composition of the serum proteins of mice immunized with adsorbed Cellophane toxoids (dotted line—fractional composition; solid line—after double immunization).

The results set out in Table 1 (and also in Fig. 1) show that during the process of immunization of the mice there was a considerable rise in the  $\gamma$ -globulin fraction, by about 2 times after a single dose and 2½ times after 2 doses. There was also a slight increase in the  $\beta$ -globulin fraction. The albumin-globulin ratio, which was 1.33 in the unimmunized mice, fell to 0.86 after 1 dose and to 0.64 after 2 doses.

A more detailed study of the changes in the serum protein fractions on immunization with type A *Clostridium botulinum* toxoids was made in experiments on guinea-pigs. The guinea-pigs (10 animals for each part of the experiment) were immunized with a single dose of 1 ml of crude or adsorbed Cellophane or normal

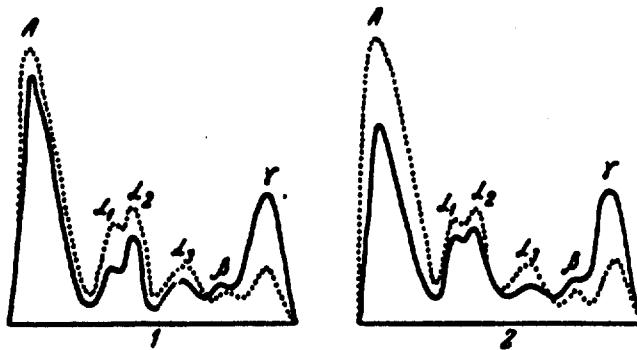


FIG. 2. Fractional composition of serum proteins of guinea-pigs: 1—immunized with normal toxoid; 2—immunized with Cellophane toxoid (dotted line—fractional composition before immunization; solid line—after immunization).

### CONCLUSIONS

- (1) Changes in the fractional composition of the serum proteins of mice and guinea-pigs during the process of immunization can be studied by electrophoretic fractionation on filter paper.
- (2) During the process of immunization of mice with cellophane type A C. *lambdacidae* toxoids there was an increase in the content of inert antigenic fractions in the serum.
- (3) During the process of immunization of guinea-pigs with both normal and Cellophane type A toxoids there was an increase in the content of  $\gamma$ -globulin and  $\beta$ -globulin protein fractions in the serum.
- (4) The immunological activity of the toxoids could not be assessed by the changes in the fractional composition of serum proteins occurring during the process of immunization, since these changes reflected the effects both of the specific antigens in the preparation and of the inert antigenic fractions present in the toxoids.

Translated by J. J. OLIVER

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## A NEW SPECIES OF BACTERIUM OF THE GENUS SHIGELLA\*

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In recent years new strains of *Shigella* have been described which do not fit into the existing classification, as a result of which many authors assign them to the so-called "atypical" group. Our investigations have shown (Manolov, 1956) that a considerable proportion of these atypical strains are the product of regressive variation under the influence of the effects of phage. They are phage-resistant strains, which have lost their agglutinability and virulence; some have undergone changes in biochemical activity and others have become yellow variants. Some atypical strains of *Shigella* are formed in the body of the patient under the influence of treatment and immuno factors in the macro-organism. These atypical strains, as we have been able to show experimentally by the keratoconjunctival reaction in guinea-pigs, have irreversibly lost their virulence and have become regressively altered forms (Manolov, 1957). However, new species may be found

\* Zh. mikrobiol., epidemiol. immunobiol. No. 12, 98-101, 1958.